Oral Immunization of Humans with *Streptococcus sobrinus* Glucosyltransferase

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The effect of oral administration of glucosyltransferase (GTF) from *Streptococcus sobrinus* 6715 on levels of immunoglobulin A (IgA) antibody to GTF in parotid saliva and on the number of indigenous *Streptococcus mutans* in the whole saliva was studied in young adult males. GTF combined with aluminum phosphate (AP) was administered in capsules to 14 subjects, while sodium phosphate buffer combined with AP was administered in the same way to 11 control subjects. Thirteen administrations were given during the first immunization regimen, and five administrations, approximately 3 months later, constituted the second immunization regimen. All subjects were given professional dental prophylaxis immediately prior to each immunization. Each subject served as his own control by using antibody and bacterial data collected prior to antigen administration for comparison. After the first immunization regimen, the GTF vaccine group exhibited a significantly higher distribution ($P < 0.05$) of normalized parotid saliva IgA antibody elevations than observed in the placebo group. Between the first and second immunization regimens a significant increase ($P < 0.05$) in parotid salivary anti-GTF activity also occurred in the GTF vaccine but not the placebo group. No significant differences between these two groups were observed on any occasion when serum IgG or IgA antibody to GTF was analyzed. Comparison of the group mean log ratios (post- to prevaccine administration) of *S. mutans* to total streptococci in whole saliva revealed that the GTF vaccine group values were always lower than those of the placebo group. These differences reached significance ($P < 0.01$) on three of the last four sampling occasions (days 21, 35, and 42) following initiation of the first immunization regimen. The mean log ratios of the GTF vaccine group were also lower than those of the placebo group after the second immunization regimen but did not reach significance. These data indicate that oral administration of GTF from the mutans streptococci has the potential to elicit a salivary IgA antibody response when combined with an aluminum-based adjuvant and that this response can interfere with the reaccumulation of indigenous *S. mutans* following dental prophylaxis.

Experiments in animals have shown that immunization with killed *Streptococcus mutans* cells or defined antigens such as glucosyltransferase (GTF), antigen A, or antigen I/II prior to infection with cariogenic *S. mutans* often significantly reduces the amount of caries and level of infection caused by these organisms. Reductions in experimentally induced dental caries have been observed after passive administration of antibody to *S. mutans* antigens in food or drinking water (16, 22) or after active immunization procedures. Antigen has been injected (3, 13, 17, 19, 29, 30) or administrated orally to animals in drinking water (21) or placed directly into the oral cavity (26). These immunization procedures favor the formation of salivary immunoglobulin A (IgA) antibody to the immunizing antigen and were accompanied by significant reductions in caries.

Investigations of the feasibility of a dental caries vaccine in humans have focused on the effect of oral administration of killed *S. mutans* cells on the formation of salivary antibody to this organism or on the effect of immunization on the indigenous or superinfecting *S. mutans* flora. Oral administration of antigen would preferentially stimulate a secretory immune response (20, 21, 26) as well as avoid the potential for formation of serum antibody, which may have host-cross-reactive properties (12). However, in the experiments performed thus far, salivary IgA antibody to *S. mutans* has been detected only occasionally after immunization (6, 20). Despite the inconsistency of detection of an antibody response, effects on the colonization of *S. mutans*

have been suggested more often (5, 6, 9; C. Y. Bonta, R. Linzer, F. Emmings, R. T. Evans, and R. J. Genco, J. Dent. Res. **68**:143, 1979).

GTFs are extracellular enzymes which synthesize water-soluble and water-insoluble glucans from sucrose. These glucans have been implicated in the plaque-forming potential of cariogenic *S. mutans*. This potential has been modified in vitro (31) with antibody to GTF. The presence of antibody of this specificity was associated in rodent models with significant reductions in dental caries caused by the mutans streptococci (*S. mutans*, *S. cicretus*, or *S. sobrinus* [25, 26, 30]). Similar reductions after immunization with GTF have also been observed in some (2) but not all (3) experiments with the primate model. No antibody reactive with human tissue was observed following either oral (26) or systemic (30) immunization with *S. mutans* GTF in adjuvant.

The successful use of GTF as an antigen eliciting a caries-protective response in rodents led us to test it in humans. Accordingly, in the experiment described herein, GTF prepared from *S. sobrinus* 6715 was administered orally to a group of young adult males for approximately 2 weeks. The purpose of this oral immunization regimen was to evaluate the effect on induction and secretion of salivary antibody to the immunizing antigen and reaccumulation of indigenous *S. mutans* after dental prophylaxis.

MATERIALS AND METHODS

Preparation of GTF. The GTF selected for the vaccine was prepared from *S. sobrinus* 6715 (serotype g) by affinity chromatography and gel filtration techniques which have

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been described previously (10, 24). This GTF vaccine had been approved by the Food and Drug Administration Bureau of Biologics for use in the present experiment under Investigational New Drug application BB-IND 1984. Organisms were grown anaerobically (10% CO_2, 90% N_2) for 24 h at 37°C in chemically defined medium (S. Socransky, C. Smith, and A. Manganello, J. Dent. Res. **52**:88, 1973). Cell-free supernatants obtained by centrifugation at 13,700 × g were brought to pH 6.5 and made 0.02% with respect to sodium azide. This supernatant was then incubated with Sephadex G100 (Pharmacia Fine Chemicals) for 2 h at room temperature. After washing the Sephadex-GTF mixture, the enzyme was eluted with an equal volume of 6 M guanidine hydrochloride (1 h). After removal of the Sephadex, the GTF-containing eluate was filtered on a column of Sepharose 4B-CL in 3 M guanidine hydrochloride. The GTF activity eluted as a single peak and was pooled, dialyzed (0.02 M sodium phosphate, pH 6.5), and filtered (Acrodiscs; Gelman Sciences) into sterile 50-ml vaccine bottles and stored frozen at −20°C until use. On each day of vaccine administration, one sterile GTF preparation was mixed with sterile aluminum phosphate (AP; Lederle Laboratories) in a laminar flow hood. Each dose contained 0.5 mg of GTF protein and 6.7 mg of AP. GTF prepared in this way synthesized more than 90% of water-insoluble glucan after 2 h of incubation with 5% sucrose and had a sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile of three to four bands which stained with Commassie blue, all of which were associated with glucan-forming activity.

The placebo preparation contained equal volumes of 0.02 M sodium phosphate and AP. These sterile solutions were mixed under laminar flow conditions and were also prepared to contain 6.7 mg of AP per dose.

Antibody and immunoglobulin analysis. Serum and parotid gland saliva samples were tested for antibody activity to GTF by the modified enzyme-linked immunosorbent assay (ELISA) technique described previously (10). Polystyrene microtiter plates coated with 0.5 μg of strain 6715 GTF per well were then incubated (2 h) at room temperature with 1:4 diluted parotid saliva (for IgA), 1:25 diluted serum (for IgA), or 1:100 diluted serum (for IgG). This was followed by development with the appropriate rabbit anti-human alpha- or gamma-chain reagent (1:200 dilution; Calbiochem-Behring) (2 h) and incubation (16 h) with 1:200 diluted alkaline phosphatase-conjugated goat anti-rabbit IgG (Miles Laboratories). After exposure to 1 mg of p-nitrophenyl-phosphate per ml, reactions were stopped after 30 min with 0.05 M NaOH. Antibody activity was compared with a positive reference standard curve constructed from 405-nm absorbances (Artek ELISA reader) of dilutions (log_2 transformed) of an ammonium sulfate-precipitated pool of parotid saliva samples containing elevated IgA antibody activity. Negative controls consisted of saliva or serum which had been repeatedly adsorbed with washed, sucrose-grown *S. sobrinus* cells.

All parotid saliva and serum samples were examined for salivary IgA antibody activity to *S. sobrinus* 6715 GTF of the same lot used for immunization. All parotid samples from one individual were always tested on the same plate, including those obtained during the screening and on day −2.

An adaptation of the technique of Mancini et al. (18) was used to assess the levels of IgA and IgG as described previously (10).

Bacterial isolation and enumeration. Approximately 2 to 3 ml of whole saliva, expectorated into a 15-ml tube, was immediately sonified (Bronson sonicator; full amplitude for
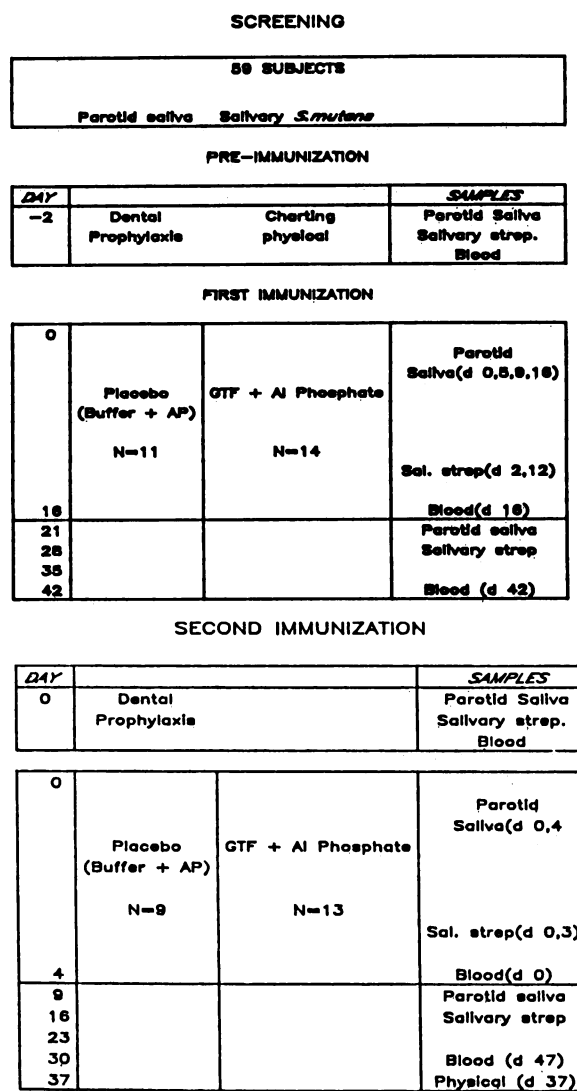

FIG. 1. Protocol for the clinical trial. Abbreviations: strep., streptococci; d, day.

10 s) while on ice. Whole saliva was then diluted in 10-fold dilution steps, using 1/4-strength Ringer solution. Plating (0.1 ml) was done in duplicate on mitis-salivarius (MS) agar for total streptococci (final dilution steps, 2×10^4, 2×10^5, and 2×10^6) and on MS agar with bacitracin (11) for *S. mutans* (i.e., mutans streptococci) (final dilution steps: 2×10^2 and 2×10^3). The number of CFU was counted after 2 days of incubation in 10% CO_2–90% N_2 at 35°C. The resulting counts were log transformed, and *S. mutans* CFU are expressed as a proportion of the total streptococcal CFU to reduce sampling variation. The log ratios obtained after immunization began were compared with the log ratios obtained in the preimmunization sampling (Fig. 1).

To identify the biotype of the *S. mutans* (i.e., mutans streptococci) recovered on the MS-bacitracin agar, colonies representing each different colonial morphology were picked for each subject. These colonies were then subcultured in Todd-Hewitt broth, plated on blood agar to confirm purity, and recultured in Todd-Hewitt broth. The cultures were then analyzed in a battery of biochemical tests in 96-well plates with the MIC-2000 (Dynatech Laboratories). The biotype

TABLE 1. Individual subject data

Group and patient no. (age in yr)	Prior to first immunization			After first immunization	
	No. of DMFS[a]	Initial S. mutans level[b] (10^3 CFU/ml)	Initial IgA level (EU ± SD)[c]	% Reduction on day 42[d]	Highest IgA antibody ratio (post vs pre)[e]
GTF vaccine					
42 (35)	25	<1	5 ± 4	—[f]	0.6
38 (18)	8	137	13 ± 7	0	2.4
47 (26)	12	315	8 ± 4	51	1.1
27 (21)	8	6	3 ± 1	83	2.9
44 (27)	15	313	300 ± 356	20	4.7
2 (35)	33	2,884	15 ± 6	27	3.6
17 (22)	19	282	105 ± 157	59	0.1
25 (33)	45	<1	3 ± 1	—	2.8
51 (19)	6	12	256 ± 380	67	3.4
34 (20)	14	4	62 ± 79	0	1.1
35 (18)	21	173	20 ± 6	89	1.1
28 (24)	8	<1	46 ± 19	—	0.8
50 (20)	17	50	56 ± 58	0	4.7
36 (19)	21	332	226 ± 203	17	1.9
Placebo					
37 (18)	0	1	42 ± 90	0	0.3
46 (18)	11	<1	7 ± 2	—	1.3
43 (22)	22	316	11 ± 12	22	0.9
41 (28)	4	14	38 ± 35	0	0.3
32 (18)	8	4	17 ± 15	0	2.7
14 (26)	2	1	21 ± 16	0	2.2
15 (36)	32	90	88 ± 106	0	0.1
24 (19)	4	13	39 ± 50	0	0.8
45 (18)	14	78	1.174 ± 422	0	0.7
1 (22)	24	1,894	238 ± 135	0	0.7
29 (18)	13	100	51 ± 49	0	0.6

[a] DMFS, Decayed, missing, and filled dental surfaces.

[b] Mean total CFU recovered on MS-bacitracin agar from whole saliva prior to the first immunization phase.

[c] Mean EU ± SD of IgA antibody activity for six parotid saliva samples from each subject, with S. sobrinus GTF (strain 6715) as the antigen. All samples were taken prior to initiation of oral immunization.

[d] Percent reduction of S. mutans/total streptococcal CFU obtained on day 42 of the first immunization phase compared with the S. mutans/total streptococcal CFU obtained initially (preimmunization). Groups significantly different at $P < 0.01$ (Mann-Whitney U test).

[e] Ratio of IgA antibody EU obtained during the first immunization phase (post) to the mean IgA EU plus 1 SD for the six preimmunization antibody determinations (pre) for each subject. Groups significantly different at $P < 0.05$ (Mann-Whitney U test).

[f] —, Only occasionally detectable levels of S. mutans in whole saliva throughout the experiment.

was deduced by comparison with the patterns devised by Shklair and Keene (23). Twenty-two subjects had biotype I S. mutans. This finding was expected since this biotype is the most prevalent among North American and Western European populations. Four subjects had at least two different biotype I strains of S. mutans (differentiated on the basis of colonial morphology). Three subjects had biotype I and biotype IV strains of S. mutans (i.e., S. sobrinus). Four subjects had only occasionally detectable levels of S. mutans in their whole saliva ($>10^2$/ml). When this occurred, a CFU value of 100 was assigned for subsequent calculations. Seven subjects, four placebo and three GTF-immunized, had multiple strains of S. mutans.

Subjects. Young adult males ($n = 59$) were initially screened for salivary antibody activity to GTF preparations. The initial intent was to select only subjects who had low preexisting levels of antibody to the GTF antigen to be used in the vaccine (S. sobrinus 6715). However, because the screening process or the physical examination revealed disqualifying factors for the study, several candidates were not included. The criterion used for assigning the remaining 25 subjects to the vaccine (GTF) or placebo group was based on the mean IgA antibody activity to S. sobrinus 6715 GTF calculated for the six parotid saliva samples obtained from each subject during the screening process. Subjects were matched with respect to their preexisting parotid saliva

antibody activity to GTF and randomly assigned to each group so that both groups had a nearly identical distribution of preexisting antibody activity to the antigen in the vaccine (Table 1). The vaccine group was assigned three more subjects than the placebo group.

Subjects were also excluded who had a history or family history of rheumatic fever or kidney disease, a heart murmur, a suspected allergy to the vaccine, or a suspected immunological impairment. All male subjects who otherwise qualified for the study were given physicals at the Lane Health Center, Northeastern University, Boston. This included demographic data, medical history, physical examination, and evaluation of blood and urine samples. Blood and urine specimens were evaluated at Bioran, Cambridge, Mass. Physicals and clinical laboratory testing were performed at the beginning and the end of the study. Daily temperature measurements and recording of adverse reactions were performed during the immunization periods. No consistent reactions or changes in clinical parameters were recorded which could be associated with receiving the vaccine or placebo during the first or second vaccine administration.

Experimental protocol. The clinical trial was carried out in four sections: screening, preimmunization, first immunization, and second immunization. During the screening section, S. mutans was detected in either the saliva or plaque of

all the subjects in the present study. However, only whole-saliva samples served as the basis for bacterial enumeration in the other sections. Figure 1 presents the schedule for vaccine administration and sample collection for these four sections. The second immunization portion began 47 days after the final sampling occasion of the first immunization period (day 42).

At preimmunization, each subject was given professional dental prophylaxis and the decayed, missing, and filled surfaces were recorded (Table 1). Parotid saliva was collected with a modified Curby cup for approximately 10 min while subjects sucked on sour-lemon drops. Fluids were centrifuged at 10,000 rpm and stored at $-20°C$. These and subsequent collections were made from the same gland for each subject.

From 5 to 10 ml of blood was also collected from the antecubital sinus of each subject by a trained phlebotomist. Blood was allowed to clot overnight at 4°C, and serum was removed after low-speed centrifugation and stored frozen at $-20°C$. The serum IgA and IgG antibody mean values measured in duplicate by ELISA from this preimmunization time were also taken as the baseline values.

In the second immunization portion of the experiment, the bacterial CFU, salivary antibody, and serum antibody values obtained from the collections prior to the administration of vaccine were taken as the baseline values.

Vaccine administration. Subjects were scheduled for immunization and fluid collections throughout the working day. However, every attempt was made to schedule each subject at the same time each day to minimize physiological variation. The days of immunization are shown in Fig. 1. Individual doses of vaccine or placebo were prepared by sterile placement of 0.8 ml of vaccine or placebo into no. 000 gelatin capsules and given to the subject to swallow with water. While the subject was waiting for his capsule, his temperature was recorded and adverse reactions, if any, were reported.

Thirteen administrations were given in 17 days in the first sequence. Eleven and a half weeks later, five immunizations were given in 5 days as the second sequence. All immunizations and antibody measurements were performed with the same lot of GTF (HS no. 1, lot 1).

Statistical analyses. Since a broad range of levels of pre-existent antibody to strain 6715 GTF existed in both groups (Table 1), the antibody or bacterial levels observed after the start of immunization of each subject were compared with that subject's own antibody or bacterial levels prior to the beginning of the trial. The mean and standard deviation (SD) of the IgA ELISA values in the six parotid saliva samples obtained before immunization for each subject were calculated and used to identify meaningful elevations in salivary antibody activity during the primary immunization portion of the protocol. Thus, an IgA antibody level to 6715 GTF was considered positive if it exceeded the mean plus the SD of the IgA antibody levels in the six samples taken before immunization of the respective subject began. Similarly, the log-transformed S. mutans/total streptococcal CFU ratio obtained in the preimmunization sampling served as the baseline for bacterial comparisons. Ratios of postimmunization to baseline data were then compared by Mann-Whitney U analysis and one- and two-way analyses of variance (ANOVAs).

RESULTS

Salivary IgA antibody. The parotid saliva samples collected after initiation of the first immunization regimen were
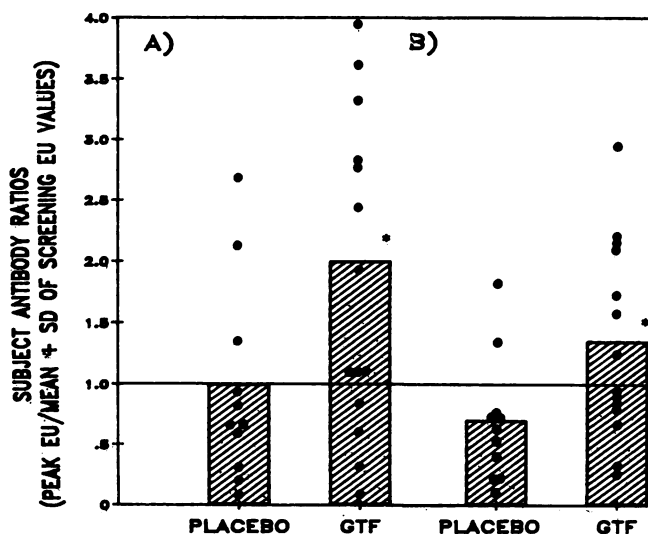

FIG. 2. Parotid salivary IgA antibody levels (EU) to *S. sobrinus* 6715 GTF during and after the first immunization regimen. Data are expressed as the ratio of antibody EU obtained after the initiation of oral immunization to the mean plus 1 SD for six parotid saliva samples taken prior to immunization. Circles represent antibody data for individual subjects. (A) Highest ratios for each individual subject in the placebo and GTF-immunized groups; (B) mean of the three highest ratios for each subject during the 42-day period after initiation of immunization. Bars represent means. The asterisks indicate statistically significant higher ratios in the GTF-immunized group ($P < 0.05$, Mann-Whitney U test).

tested for IgA antibody activity to the immunizing antigen. Only 3 of 11 (27%) subjects in the placebo group had IgA antibody levels to 6715 GTF which exceeded the values obtained during screening at any time during the first immunization portion of the experiment. Of the GTF vaccine group, 10 of 14 (71%) subjects had IgA antibody levels to the antigen which exceeded their screening values. The highest IgA antibody activity ratio for each subject at any time during the primary immunization phase is shown in Fig. 2A. The ratios observed for the GTF vaccine group were significantly ($P < 0.05$, Mann-Whitney U test) higher than those obtained for the placebo group. The three highest ratios detected among the seven saliva samples collected from each subject in the first immunization phase were also calculated as a method of minimizing the potential distortion of the data by single aberrant values and as a method of dealing with the potentially complex response kinetics (Fig. 2B). Again, the GTF vaccine group had a significantly higher distribution of IgA antibody levels ($P < 0.05$) than did subjects receiving the placebo.

In general, the antibody levels of most subjects were higher in the second immunization samples than in the first immunization samples. The GTF vaccine group values tended toward higher ratios with greater frequency than the placebo group values whether expressed as peak IgA antibody response (Fig. 3A) or as mean of the three highest data (Fig. 3B). The change in peak IgA antibody ELISA unit (EU) ratios of the placebo group between the first and second immunization regimens was not significant (1.0 ± 0.2 versus 3.6 ± 2.5; mean EU ratio $\pm$ standard error). However, change in the respective antibody values in the GTF vaccine group (2.0 ± 0.3 versus 17.0 ± 7.3) was significant ($P < 0.05$, one-way ANOVA).
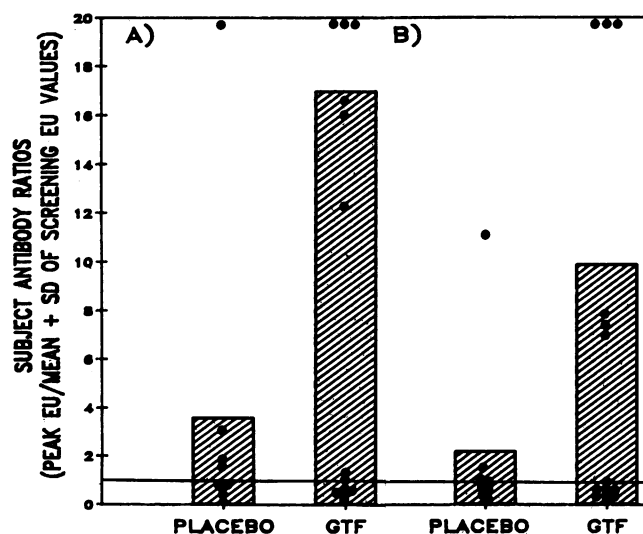
FIG. 3. Parotid salivary IgA antibody levels (EU) to *S. sobrinus* 6715 GTF during and after the second immunization regimen. Data are expressed as the ratio of antibody EU obtained after the initiation of oral immunization to the mean plus 1 SD for six parotid saliva samples taken prior to all immunizations. Circles represent (A) the highest ratios for each individual subject in the placebo and GTF-immunized groups or (B) the mean of the three highest ratios for each subject during the 37-day period after initiation of immunization. Bars represent means.
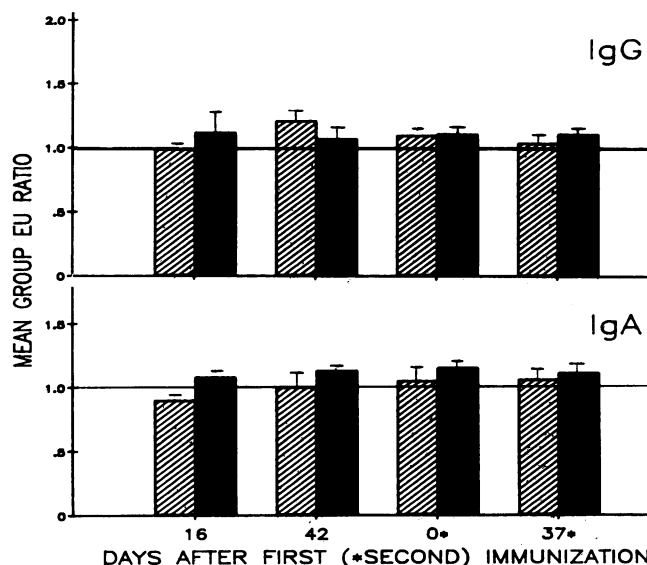

FIG. 4. Serum IgG and IgA antibody levels (EU) to *S. sobrinus* 6715 GTF during the first (days 16 and 42) and second (days 0* and 37*) immunization regimens. Data are expressed as the ratio of antibody EU obtained after initiation of the first oral immunization to the antibody EU of the sera taken at the time of the initial dental prophylaxis. Individual ratios were averaged for each group. Brackets enclose 1 standard error. The horizontal lines represent preimmunization serum antibody activity. Hatched bars, GTF group; solid bars, placebo group.

Serum IgG antibody. Blood was collected on the day of the first dental prophylaxis (day −2 of the first immunization collection phase), day 16, day 42, the day of the second dental prophylaxis (day 0, second immunization collection phase), and 37 days after the beginning of the second immunization phase. The sera were tested for IgG and IgA antibody to the immunizing GTF antigen, and data were compared with preimmunization serum antibody levels. The mean ratios for each group on each collection day are shown in Fig. 4. No significant differences within or between the groups were observed on any occasion for either serum immunoglobulin isotype.

Bacterial studies. Mutans streptococci in saliva were expressed as a proportion of the total streptococci to monitor the selective effect which antibody to *S. sobrinus* GTF, induced by the vaccine, might have had on indigenous organisms. Prior to immunization the percentages of *S. mutans* among the total streptococci were nearly identical in both groups (mean, 0.0111% in the placebo group, 0.0114% in the GTF-immunized group). The *S. mutans*/total streptococci values obtained after initiating the first oral immunization regimen were compared with the data obtained prior to the first dental prophylaxis by the use of log ratios. The log ratios for each group on every sampling occasion were averaged for both groups (Fig. 5), and analysis of group data on individual days revealed that the GTF-immunized group was significantly lower (Mann-Whitney U test) on three of the last four sampling occasions (days 21, 35, and 42). Furthermore, the mean ratios of the GTF vaccine group were always lower than those of the placebo group. Two-way ANOVA of the mean values for the two groups over the 40-day sampling period revealed that the GTF vaccine group data were significantly lower than the placebo group data for the course of the primary immunization ($P < 0.02$). No significant differences in total streptococcal CFU ratios

(post- versus preimmunization) alone were ever observed between the groups.

The *S. mutans*/total streptococci log CFU data obtained after initiation of the second oral immunization regimen were compared with those data obtained immediately prior to the
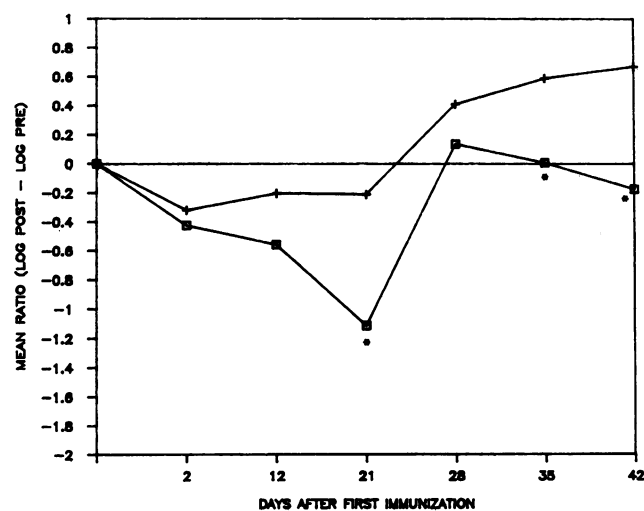

FIG. 5. Comparisons of the proportions (log ratios) of *S. mutans* in the total streptococci present in whole saliva before (pre) with the respective proportions (log ratios) present after (post) the initiation of the first oral immunization are presented for the GTF-immunized (□) and placebo (+) groups. All CFU values were log transformed before analysis. The means of individual-subject log ratios (log post to log pre) were determined for each group on each sampling day. Asterisks indicate days on which differences between group data were significant by the Mann-Whitney U test (day 21, $P < 0.001$; day 35, $P < 0.01$; day 42, $P < 0.01$).
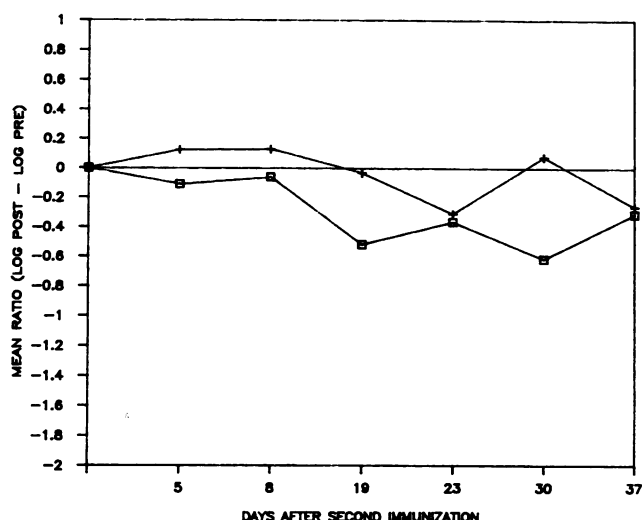
FIG. 6. Comparisons of the proportion of *S. mutans* to the total streptococci in whole saliva before and after the initiation of the second immunization regimen for the GTF-immunized (□) and placebo (+) groups. All CFU values were log transformed before analysis. Data are expressed as mean log ratios (log post to log pre) for each group on each sampling day.

second dental prophylaxis. The mean log ratios (post- versus pre-second vaccine administration) of the GTF-immunized group were lower than the placebo groups' log ratios at every sampling occasion, although these differences did not reach statistical significance on individual days (Fig. 6). Neither group demonstrated the increase in ratios seen at the later sampling times of the first immunization portion of the experiment (Fig. 5). During the course of the experiment, no consistent variation occurred in the distribution of *S. mutans* strains which had different colonial morphologies in any subject.

DISCUSSION

Oral administration of *S. sobrinus* 6715 GTF in AP over a 2-week period gave rise to a modest salivary IgA antibody response to this antigen in a significant number of young adult males. This is the first demonstration of a secretory IgA antibody response in humans to a defined antigen from the mutans streptococcal group. Hughes et al. (14) reported that subjects subcutaneously injected with a cell wall antigen (antigen A) showed a serum IgG antibody response. However, the response in saliva was not investigated. All other modern studies in humans have used intact, killed *S. mutans* cells orally administered in capsules (5, 6, 20; C. Y. Bonta, R. Linzer, F. G. Emmings, R. T. Evans, and R. J. Genco, J. Dent. Res. **68**:143, 1979) or in solution (9) or topically applied to the oral mucosa (15). With two exceptions (6, 20), these immunization regimens, which varied from 3 to 14 doses, did not induce a detectable salivary IgA response to the immunizing antigen. The successful demonstration of a salivary antibody response in the present study may be attributed to the use of a vaccine containing concentrated amounts of an immunogenic, defined antigen combined with AP, previously shown to be an effective adjuvant for secretory immune responses in animals (7). The use of this adjuvant may enhance the uptake of antigen by accessory cells such as M cells in the gut-associated lymphatic tissue. Immunization of larger subject populations, together with

the use of each individual as his own control for analysis of change in antibody levels, also enhanced the potential for the observation of meaningful differences.

The mean parotid salivary IgA antibody levels to the *S. sobrinus* 6715 GTF showed a modestly positive ($r = 0.362$; $P < 0.05$) correlation with the numbers of *S. mutans* recovered in whole-saliva samples of the study population. This was consistent with our previous observation that parotid saliva samples from young adult subjects with high caries experience contained significantly more IgA antibody to the *S. mutans* GTF than did those from caries-free subjects (M. S. Block, D. J. Smith, J. L. Ebersole, and M. A. Taubman, J. Dent. Res. **58**:145, 1979). These observations indicated that the host was responding to the greater indigenous antigenic challenge by forming increased levels of salivary antibody to the mutans streptococci. Antibody occurring in response to infection with *S. mutans* may play an important role in modifying the colonization and cariogenicity of these organisms (9). These observations also suggested that a response to an orally administered vaccine containing one of these antigens might occur.

We anticipated that the subjects with the lowest levels of *S. mutans* infection or the lowest levels of salivary IgA antibody to the GTF antigen would demonstrate the most marked changes in salivary IgA antibody to the immunizing antigen after oral administration of the vaccine (32). However, no significant correlations were found in the immunized group between the changes in antibody levels and (i) initial antibody level, (ii) initial concentrations of *S. mutans* in whole saliva, (iii) caries experience, or (iv) caries activity (Table 1). This could simply have been the result of an insufficiently large test population. On the other hand, the extent and duration of exposure to *S. mutans* antigens were quite different among these subjects, as was the number of restored caries and the range of salivary *S. mutans* levels observed. This nonuniformity in previous exposure to *S. mutans* antigens is likely to result in nonequivalent stages of immunological memory to vaccine epitopes among subjects in the present study. The diversity in antigenic experience encountered in an adult population could be partially overcome by testing this vaccine in a population not yet exposed to *S. mutans*-specific epitopes, e.g., young children (4, 10).

The most consistent effect of oral administration of the GTF vaccine was retarded reaccumulation of the indigenous *S. mutans* following dental prophylaxis. The presence of salivary antibody to the strain 6715 GTF has been shown to interfere with the accumulation and permanent colonization of *S. sobrinus* (28) as well as other biotypes of mutans streptococci in rodents (25). Antibody-mediated effects on colonization or accumulation may occur through interference with the glucan-forming activity of GTF, creating fewer binding sites for *S. mutans* in plaque (31). Elimination of *S. mutans* from the oral cavity by swallowing GTF-bearing cells which were aggregated by salivary antibody may also have modified the reacquisition of indigenous *S. mutans* in vaccinated individuals. These effects on indigenous accumulation occurred even in immunized subjects whose salivary IgA antibody levels did not exceed the mean plus the SD of the antibody values obtained during the screening phase. Similarly, in other experiments in which intact *S. mutans* cells were used as the antigen, changes in bacterial levels rather than antibody levels predominated (5, 9; C. Y. Bonta, R. Linzer, F. G. Emmings, R. T. Evans, and R. J. Genco, J. Dent. Res. **68**:143, 1979). These observations may result from the fact that the assays used may not always be sensitive enough to distinguish moderate changes in salivary

antibody levels which may be sufficient to have overt effects on bacterial populations. The effect of changes in levels of specific antibody may also be augmented by other components of the host antibacterial network (1).

Secondary salivary IgA antibody levels in the parotid saliva samples of all subjects showed more variation than was seen after the first immunization, although the GTF-immunized group was still clearly higher. Furthermore, salivary antibody levels were elevated in both groups (Fig. 3). A possible contributing factor to the overall elevation in antibody levels may have been the result of an immune response to the two dental prophylaxes performed as part of the protocol. It is possible that since the teeth of nearly everyone in the study harbored *S. mutans*, which produce GTF and bear surface GTF, a thorough cleaning could have resulted in transitory immunization (8). The general presence of higher antibody levels during the second immunization regimen in both groups may also have contributed to the observation that the proportion of *S. mutans* to total streptococci after the second prophylaxis and oral immunization did not demonstrate the increases observed in the latter half of the first immunization portion of the experiment.

The results of this study suggest that oral administration of GTF from the mutans streptococci has the potential to elicit a salivary IgA antibody response when combined with an aluminum-based adjuvant. In addition, oral immunization with this antigen was associated with interference with repopulation of the oral cavity by *S. mutans*. While these effects were relatively short-lived, efforts to modify the antigen dose, frequency of administration, composition, route of administration, or presentation of the antigen to appropriate antigen-presenting cells may significantly increase the intensity and duration of the response. Furthermore, administration of an oral vaccine to an immunologically naive population may also enhance the immune response. Such a strategy might prevent disease since it would provide an immune barrier prior to infection rather than superimposing one on preexisting flora.

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LITERATURE CITED

1. Arnold, R. R. 1986. Innate immunity and *Streptococcus mutans*, p. 297–306. *In* S. Hamada, S. M. Michalek, H. Kiyono, L. Menaker, and J. R. McGhee (ed.), Cellular, molecular and clinical aspects of *Streptococcus mutans*. Elsevier Publications, Amsterdam.
2. Bahn, A. N., I. L. Sklair, and J. A. Hayashi. 1977. Immunization with dextransucrases, levansucrases and glycosidic hydrolases from oral streptococci. II. Immunization with glucosyltransferases, fructosyltransferases and glycosidic hydrolases from oral streptococci in monkeys. J. Dent. Res. 56:1586–1598.
3. Bowen, W. H., B. Cohen, M. F. Cole, and G. Colman. 1975. Immunization against dental caries. Br. Dent. J. 139:45–58.
4. Carlsson, J., H. Grahnen, G. Jonsson, and S. Wikner. 1975. Lactobacilli and streptococci in the mouth of children. Caries Res. 9:333–339.
5. Cole, M. F., C.-G. Emilson, S. D. Hsu, S.-H. Li, and W. H. Bowen. 1984. Effect of peroral immunization of humans with *Streptococcus mutans* on induction of salivary and serum antibodies and inhibition of experimental infection. Infect. Immun. 46:703–709.
6. Czerkinsky, C., S. J. Prince, S. M. Michalek, S. Jackson, M. W. Russell, Z. Moldovenu, J. R. McGhee, and J. Mestecky. 1987. IgA antibody-producing cells in peripheral blood after antigen ingestion: evidence for a common mucosal immune system in humans. Proc. Natl. Acad. Sci. USA 84:2449–2453.
7. Ebersole, J. L., M. A. Taubman, and D. J. Smith. 1983. Adjuvants, glucosyltransferases and caries vaccine, p. 241–248. *In* R. Doyle and J. Ciardi (ed.), Glucosyltransferases, glucans, sucrose and dental caries. (special supplement to Chemical Senses).
8. Ebersole, J. L., M. A. Taubman, D. J. Smith, and A. S. Haffajee. 1985. Effect of subgingival scaling: systemic antibody responses to oral microorganisms. Infect. Immun. 48:534–539.
9. Gahnberg, L., and B. Krasse. 1983. Salivary immunoglobulin A antibodies and recovery from challenge with *Streptococcus mutans* after oral administration of *Streptococcus mutans* vaccine in humans. Infect. Immun. 39:514–519.
10. Gahnberg, L., D. J. Smith, M. A. Taubman, and J. L. Ebersole. 1985. Salivary IgA antibody to glucosyltransferase of oral streptococcal origin in children. Arch. Oral Biol. 30:551–556.
11. Gold, O. G., H. V. Jordan, and J. van Houte. 1973. A selective medium for *Streptococcus mutans*. Arch. Oral Biol. 18:1357–1364.
12. Hughes, M., S. M. MacHardy, A. J. Sheppard, and N. C. Woods. 1980. Evidence for an immunological relationship between *Streptococcus mutans* and human cardiac tissue. Infect. Immun. 27:576–588.
13. Hughes, M., S. M. MacHardy, and A. J. Sheppard. 1983. Manufacture and control of a dental caries vaccine for parenteral administration to man, p. 259–268. *In* R. Doyle and J. Ciardi (ed.), Glucosyltransferases, glucans, sucrose and dental caries (special supplement to Chemical Senses).
14. Hughes, M., S. M. MacHardy, A. Sheppard, D. Langford, and W. Shephard. 1986. Experiences in the development of a dental caries vaccine, p. 297–306. *In* S. Hamada, S. M. Michalek, H. Kiyono, L. Menaker, and J. R. McGhee (ed.), Cellular, molecular and clinical aspects of *Streptococcus mutans*. Elsevier Publications, Amsterdam.
15. Krasse, B., L. Gahnberg, and D. Bratthall. 1978. Antibodies reacting with *Streptococcus mutans* in secretions from minor salivary glands in humans. Adv. Exp. Biol. Med. 109:349–354.
16. Lehner, T., J. Caldwell, and R. Smith. 1985. Local passive immunization by monoclonal antigen I/II in the prevention of dental caries. Infect. Immun. 50:796–799.
17. Lehner, T., M. W. Russell, J. Caldwell, and R. Smith. 1981. Immunization with purified protein antigens from *Streptococcus mutans* against dental caries in rhesus monkeys. Infect. Immun. 34:407–415.
18. Mancini, G., A. O. Carbonara, and J. P. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:335–354.
19. McGhee, J. R., S. M. Michalek, J. Webb, J. M. Navia, A. F. R. Rahman, and D. W. Legler. 1975. Effective immunity to dental caries: protection of gnotobiotic rats by local immunization with *Streptococcus mutans*. J. Immunol. 114:300–305.
20. Mestecky, J., J. R. McGhee, R. R. Arnold, S. M. Michalek, S. J. Prince, and J. L. Babb. 1978. Selective induction of an immune response in human external secretions by ingestion of bacterial antigen. J. Clin. Invest. 61:731–737.
21. Michalek, S. M., J. R. McGhee, J. Mestecky, R. R. Arnold, and L. Bozzo. 1976. Ingestion of *Streptococcus mutans* induces secretory immunoglobulin A and caries immunity. Science 192:1238–1240.
22. Michalek, S. M., J. R. McGhee, R. R. Arnold, and J. Mestecky. 1978. Effective immunity to dental caries: selective induction of secretory immunity by oral administration of *Streptococcus mutans* in rodents. Adv. Exp. Med. Biol. 109:261–269.
23. Shklair, I., and H. Keene. 1976. Biochemical characterization and distribution of *Streptococcus mutans* in three diverse populations, p. 201–210. *In* M. Stiles, W. Loesche, and T. O'Brien (ed.), Proceedings: Microbial Aspects of Dental Caries (special supplement to Microbiology Abstracts).
24. Smith, D. J., M. A. Taubman, and J. L. Ebersole. 1979. Preparation of glucosyltransferase from *Streptococcus mutans*

by elution from water-insoluble polysaccharide with a dissociating solvent. Infect. Immun. 23:446–452.

25. Smith, D. J., M. A. Taubman, and J. L. Ebersole. 1978. Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in hamsters caused by homologous and heterologous serotypes of *S. mutans*. Infect. Immun. 21:843–851.

26. Smith, D. J., M. A. Taubman, and J. L. Ebersole. 1979. Effect of oral administration of glucosyltransferase antigens on experimental dental caries. Infect. Immun. 26:82–89.

27. Smith, D. J., M. A. Taubman, and J. L. Ebersole. 1980. Local and systemic antibody response to oral administration of glucosyltransferase antigen complex. Infect. Immun. 20:441–450.

28. Smith, D. J., M. A. Taubman, and J. L. Ebersole. 1982. Effects of local immunization with glucosyltransferase on colonization of hamsters by *Streptococcus mutans*. Infect. Immun. 37:656–661.

29. Taubman, M. A., and D. J. Smith. 1974. Effects of local immunization with *Streptococcus mutans* on induction of salivary IgA antibody and experimental dental caries in rats. Infect. Immun. 9:1079–1091.

30. Taubman, M. A., and D. J. Smith. 1977. Effects of local immunization with glucosyltransferase on experimental dental caries in rats and hamsters. J. Immunol. 118:710–720.

31. Taubman, M. A., D. J. Smith, J. L. Ebersole, and J. D. Hillman. 1982. Immunological interference with accumulation of cariogenic microorganisms on tooth surfaces, p. 371–382. *In* W. Strober, L. Hanson, and K. W. Sell (ed.), Recent advances in mucosal immunity. Raven Press, New York.

32. Waldman, R. H., J. Stone, K.-C. Bergmann, V. Lazzell, A. Jacknowitz, E. R. Waldman, and S. Howard. 1986. Secretory antibody following oral influenza immunization. Am. J. Med. Sci. 292:367–371.